THE DURATION OF THE LIFE OF THE TUBERCLE BACILLUS IN CHEESE.

BY

F. C. HARRISON,

Agricultural College, Guelph, Ontario.

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Introductory.—An American student, engaged in advanced bacteriological work in Europe, in the year 1900, made an original investigation as to the life of the germs of tuberculosis when found in cheese. Circumstances caused the study to be mainly with the standard variety of cheese in Switzerland, which is known in the United States under the names Emmenthal, Gruyère, and Switzer-käse. The work attracted much favorable comment by bacteriologists of foremost rank in Europe, who accepted the results as beyond controversy.

The record being published in German only, the author kindly made an English translation at my request. Then, upon the suggestion of a possible doubt as to the applicability of his first conclusions to the Cheddar, or standard American, variety of factory cheese, Professor Harrison repeated his experiments, after his return home, under more satisfactory conditions. His original results were verified, as shown in the report which follows.

Although in portions of the report describing details of procedure the author uses the technical terms of an experimenter in bacteriology, all parts which are of popular interest are given in language free from such terms. The methods followed were those usual in such work. Virulent disease germs of the kind to be studied were separated, identified, and cultivated by themselves. Cheese was specially made into which these living germs of disease were introduced. Particles of the infected material were taken at different dates, as the cheese increased in age and maturity, and injected into the blood or tissues of small animals. Guinea pigs were used in these experiments. The animals were then watched, tested, and examined, to note the results and determine when and to what extent the disease of tuberculosis was thus transmitted to them through the medium of the cheese.

Evidence is thus presented which appears to demonstrate that if milk containing germs of the much-dreaded disease of bovine tuberculosis is made into cheese by the methods most common in this country and the living bacillus thus lodged in the cheese, these germs will all die and become harmless by the time the cheese is properly ripened and ready for use as food. In other words, this investigation shows that there is no danger of taking the living germs of consumption into the human system by eating well-cured cheese of the common kind. It is important to note that the cheese should be at least three months old and preferably four. The same degree of safety does not apply in case of younger and immature cheese, which seems to be growing in favor in some sections. This affords an additional argument for placing upon every cheese the date when made. It is an excellent custom, already practiced by many good makers.

The result of Professor Harrison's work is of great interest and importance to all consumers of cheese, as well as to dealers and manufacturers. By the courtesy of the author the record is now first printed in English and deserves to be widely disseminated.

This publication being intended to give an account of the work and its results for general information, much of the detail of original record which would belong to a strictly scientific report upon such an investigation has been omitted as being needless here.

HENRY E. ALVORD,
Chief of Dairy Division.

WASHINGTON, D. C., *March, 1902.*

REPORT OF PROFESSOR HARRISON'S ORIGINAL EXPERIMENTS IN SWITZERLAND.

[Translated from the German by the author.]

Pathogenic germs, as a rule, have but slight vitality in cheese, but of those which may be present the tubercle bacillus seems to be the most resistant. The large percentage of tuberculous dairy cattle in Europe and even in newer countries makes it important to inquire as to the length of life of the tubercle bacillus in cheese, how often and in what numbers it may be present; what danger there is to the individual who may eat cheese containing the germ, and whether the by-products from the manufacture of such cheese are dangerous to animals fed upon them.

PREVIOUS INVESTIGATIONS IN THE SAME GENERAL FIELD.

If we accept the conclusions of Rabinowitch and Klemperer,[1a] we must be prepared to acknowledge that, not only the milk of cows having either tubercle in the udder or advanced generalized tuberculosis is dangerous, but also the milk of cows which exhibit no clinical symptoms of the disease, but give the tuberculin reaction. If these results are accepted, a considerable increase must be recognized in the percentage of tuberculous cattle whose milk will be regarded as dangerous or treated as suspicious.

Galtier,[2] in 1887, examined cheese and whey to ascertain what risk of contracting tuberculosis man and animals ran from eating these products. The experiments were made with normal milk tuberculosed by the addition of morbid material taken from diseased phthisical cows killed in the abattoirs or from rabbits which had died from tuberculosis induced by injection. The milk was coagulated by the addition of rennet, and with the cheese and whey thus obtained attempts were made to ascertain the facts as to the transmission of tuberculosis. Unfortunately, Galtier does not state whether the curd was pressed and made into hard cheese or was left without pressure as soft cheese. The inoculations were made into guinea pigs (intraperitoneally) and into rabbits (intravenously). The

[a] For bibliographic references, see page 233.

particles of cheese were triturated in sterilized water, and the liquid part of the mixture, separated by decantation or filtration, was used for the inoculations. The whey was also filtered before each inoculation, and in this manner the animals were inoculated with cheese and whey of the age of 5, 10, 15, 20, and 30 days, etc. Some of the attempts did not furnish positive results; but the number of cases of undeniable transmission was large enough to establish the preservation of tubercle germs and of the noxious character of the products of the milk which contained them. Galtier obtained "generalized tuberculosis" from cheese not salted and salted, 5, 10, and 15 days old, and from some which was even 2 months and 10 days old. In several experiments, the disease was found in only one-half or one-third of the subjects inoculated; and in other experiments, which were not the most numerous, the results were negative in animals inoculated with cheese 2 months old and even only 15 days old.

The whey, separated from cheese 5, 10, and 15 days before, agitated and filtered before inoculation, invariably gave tuberculosis to guinea pigs, which, however, resisted the inoculation of this product made in doses of 2 c. c.

Like results were obtained with rabbits. Whey 2 days old produced innumerable tubercle lesions in the rabbits inoculated; and the same product, kept for 9 and 16 days, equally produced the disease, but in a slower and milder form.

Finally, cheese 5, 9, 16, and 20 days old, produced authentic tuberculosis in rabbits, from which the germ was subsequently cultivated on artificial media and transmitted to other animals.

From these experiments Galtier concludes that coagulated milk, fresh cheese, and salted cheese, made from the milk of tuberculous cows, may infect man; that the by-products from such milk fed to swine and farmyard fowls may infect these animals; and that it is not irrational to conclude that a certain number of the cases of chicken and swine tuberculosis are due to this cause.

Heim[3] in 1889 also gave attention to this subject. He used cultures of tubercle bacilli grown upon sheep's blood serum. In curd which was mixed with tubercle bacilli he found living germs on the second day (using guinea pigs, injected intraperitoneally) with water-suspended particles of the curd, but none after 14 days.

In curd prepared from milk to which tubercle bacilli had been added, germs were found, even after 14 days, but none after 4 weeks; and also in the whey obtained at the same time, the bacilli were found alive after 14 days.

As may be noted, these results of Galtier's and Heim's differ considerably, the difference being accounted for in part by the different methods of experiment; and for this reason, as well as others, it seemed advisable to make some further investigation as to the duration of the life of the tubercle bacillus in cheese.

DESCRIPTION OF METHODS EMPLOYED IN SWITZERLAND.

While studying at Berne, a series of experiments was planned and conducted as follows:

Cultures used.—The growth from five potato-tube cultures, 4 weeks old, was scraped off and triturated in a sterile mortar with 6 per cent glycerin. To this mass was added the surface growth of tubercle bacilli from 125 c. c. of glycerin bouillon. The whole quantity was ground up as finely as possible and sterilized water (about 200 c. c.) added.

Manufacture of the cheese.—Two cheeses were made in separate vessels and by somewhat different methods, 10 liters, or about 22 pounds, of milk having been used for each. It was intended that these cheeses should, respectively, resemble as nearly as possible the Swiss Emmenthal and American Cheddar varieties. Both lots of milk were heated to 35° C. (95. F.), a portion of the tuberculous emulsion was added to each, and the mixture was thoroughly stirred. One-fifth of one of Hansen's rennet tablets, dissolved in water, was used for each cheese. Five minutes after the addition of the rennet, more of the tubercle emulsion was added, and again after fifteen minutes. The same amount was used for each cheese.

The milk had completely set in thirty-three minutes, and the curd was then cut and stirred with a wire stirrer and left for ten minutes. At the expiration of this time, cheese "A" was heated to 55° to 56° C., and kept constantly stirred for thirty minutes at this temperature, thus following the practice usual in making Emmenthaler cheese. At the end of this time, the curd was transferred to a suitable mold, and a 5-kilogram weight (11 pounds) placed upon it. Cheese "B" was kept stirred for two hours at a temperature of 36° to 38° C. (97° to 100° F.); this was in accordance with the Cheddar cheese method, in which the temperature does not rise above 37° C. (or above 98° F.). On account of lack of the necessary apparatus, other processes usual in making Cheddar cheese were not followed. The curd of this cheese (B) was also transferred to a mold and a 5-kilogram weight placed upon it. After four hours, the weight was increased to 8 kilograms (17½ pounds) on each cheese. The following day the cheese was turned, an operation which was twice repeated. On the second day they were removed from the molds, and salt was rubbed all over them, a thin layer being left on the top. The cheeses were kept at a temperature of about 5° C. (41° F.) for a week, being rubbed with salt and turned every day; at the end of about 10 days they were removed to an empty chest in which the temperature was between 12° and 18° C. (53° to 65° F.). The cheeses were kept free from mold by washing them every second or third day with strong brine for four weeks, after which they were washed only once a week.

Amount of cheese used for injection.—A plug of cheese was removed

with a sterile cork borer, and a varying quantity, drawn from the center of the cheese, was transferred to a sterile mortar. This portion was thoroughly triturated with sterile distilled water or bouillon, and the remainder of the plug was replaced in the cheese.

Injections.—Guinea pigs were used as test animals, and the injections were made subcutaneously near the inguinal region. The first inoculation was very difficult, as the needle of the syringe, although large, became blocked by particles of curd. For subsequent injections the following technique was used, which gave good satisfaction: The animal was fastened to a dissecting board, and the hair was closely clipped over the inner portion of the thigh. This part was then washed with corrosive sublimate, after which a small hole was made in the skin with a large needle, and the end of a glass pipette (freshly made for each inoculation and consequently sterile) filled with the emulsion was introduced and the contents blown in. A pair of pressure forceps, immediately applied to the small hole and held for a few minutes, closed the orifice and prevented the escape of the emulsion.

The average weight of the guinea pigs used in the experiment was 500 grams, or about 18 ounces.

Observations of the animals during life.—The animals were weighed and examined every week; and the presence of tuberculosis was often indicated by the swelling of the inguinal glands and by a gradual loss of weight. At the end of six weeks or longer, .1 c. c. of tuberculin was injected, and in animals badly affected death usually followed in twenty-four hours; but in animals slightly diseased there was a marked rise of temperature, often more than 2° C. In fact, the smaller the amount of tubercle present, the more intense was the reaction. The same dose of tuberculin was often injected into healthy animals, but in no case did the temperature rise 0.4° C. above the normal.

Postmortem.—The postmortem examinations were made shortly after death, and animals not killed by the tuberculin injection were chloroformed and promptly examined. The presence of all lesions was noted. At least three preparations were made from diseased glands and organs, and these were stained by the Ziel Neelsen method.

Results of inoculating guinea pigs with cheese and whey of various ages, to test infection of tubercle bacilli.

EXHIBIT I.—EMMENTHALER CHEESE.

No. of guinea pig.	Age of inoculated cheese.	Quantity of cheese inoculated.	Tuberculin reaction.	Died or killed, and days after inoculation.	Postmortem.	Microscopic examination.	Remarks.
		Grams.	° *C.*				
1	Fresh curd	½	1.7	Died, 52 days	Tubercle in inguinal and retroperitoneal glands, mesentery, spleen, and liver.	Tubercle bacilli in the glands and spleen.	Died 24 hours after injection of 0.1 c. c. tuberculin.
2	7 days	½		Died, 2 days	Muscles dark and infiltrated, edematous fluid.	Bacilli and staphylococci in the fluid.	Cultures and inoculation of rabbits showed *B. coli*, *B. edematis maligni*, and *Staphylococcus albus*.
3	do	½		do	do	do	Do.
4	14 days	½	2.0	Killed, 56 days	Tubercle in inguinal and retroperitoneal glands, liver, and spleen.	Tubercle bacilli in glands and spleen.	Inoculated with 0.1 c. c. tuberculin.
5	do	½	2.2	do	do	do	Do.
6	21 days	½	2.0	Killed, 49 days	Tubercle in inguinal and retroperitoneal glands; spleen slightly affected.	Tubercle bacilli in the glands	Do.
7	do	½	1.9	do	Tubercle in inguinal, retroperitoneal, and mesenteric glands; spleen slightly affected.	do	Do.
8	28 days	½	1.9	Killed, 59 days	Tubercle in inguinal and retroperitoneal glands.	do	Do.
9	do	½	1.4	do	do		
10	33 days	½	2.0	Killed, 64 days	All organs normal; animal in good health; has gained 160 grams since inoculation.	No tubercle bacilli found in either the glands or organs.	Inoculated with 0.1 c. c. tuberculin. A control animal injected with the same quantity of tuberculin gave a reaction of 0.2° C.
11	do	½	2.3	do	All organs healthy; has gained 230 grams since inoculation.	do	Do.
12	40 days	½	0.3	Killed, 70 days	All organs and glands normal	No tubercle bacilli	Inoculated with 0.1 c. c. tuberculin.
13	do	½	0.4	do	do	do	Do.
14	47 days	1	0.2	Killed, 64 days	do	do	Do.
15	do	1	0.4	do	do	do	Do.

16	54 days	1	0.5	Killed; 66 days	ditto	ditto	Do.
17	ditto	1	0.3	ditto	ditto	ditto	Do.
18	63 days	1	0.3	Killed, 58 days	ditto	ditto	Do.
19	ditto	1		Died, 7 days	ditto	ditto	
20	70 days	1		Died, 46 days	Intestine strangulated through a hole in the mesentery.	ditto	
21	ditto	1	0.4	Killed, 65 days	All organs and glands normal	ditto	Do.

EXHIBIT II—CHEDDAR CHEESE.

1	Fresh curd	½	1.2	Died, 45 days	Inguinal, mesenteric, and retroperitoneal glands tuberculous. Tubercle in the spleen.	Tubercle bacilli in all glands	Died 36 hours after injection of 0.05 c. c. tuberculin.
2	7 days	1		Died, 2 days	Muscles dark and infiltrated; edematous fluid.	Large bacillus and staphylococci in the fluid.	Cultures and inoculation of rabbits showed *B. coli*, *B. edemantis maligni*, and *Staphylococcus albus*.
3	ditto	1		ditto	ditto	ditto	Do.
4	14 days	½		Died, 35 days	Inguinal, retroperitoneal, and mesenteric glands infected; also spleen and liver.	Tubercle bacilli in glands and spleen.	
5	ditto	½	1.6	Died, 49 days	ditto	ditto	Died 24 hours after injection of 0.1 c. c. tuberculin.
6	21 days	½	1.1	Killed, 49 days	Tubercle in inguinal and retroperitoneal glands, spleen, and liver.	ditto	
7	ditto	½	1.0	Died, 50 days	ditto	ditto	Do.
8	28 days	½		Died, 56 days	Tubercle in inguinal and retroperitoneal axillary glands, spleen, liver, and lungs.	Tubercle bacilli in glands and liver.	Died 1 hour after injection of 0.1 c. c. tuberculin; was very weak and sick before injection.
9	ditto	½	0.9	Killed, 57 days	Tubercle in glands, spleen, liver, and lungs. Tubercles on the peritoneum.	Tubercle bacilli in glands and spleen.	
10	33 days	½	1.2	Died, 64 days	Tubercle in glands, spleen, and liver.	Tubercle bacilli in glands	Died 24 hours after injection of 0.1 c. c. tuberculin.
11	ditto	½		Died, 10 days	Peritonitis	No tubercle bacilli in glands	
12	40 days	½		Died, 7 days	Very much wasted; large swelling at seat of inoculation.	Cultures from the spleen and microscopic examination showed staphylococcus albus.	Do.

Results of inoculating guinea pigs with cheese and whey of various ages, to test infection of tubercle bacilli—Continued.

EXHIBIT II.—CHEDDAR CHEESE—Continued.

No. of guinea pig.	Age of inoculated cheese.	Quantity of cheese inoculated.	Tuberculin reaction.	Died or killed, and days after inoculation.	Postmortem.	Microscopic examination.	Remarks.
		Grams.	° *C.*				
13	40 days	¼	0.9	Died, 70 days	Tubercle in inguinal and retroperitoneal glands, and in spleen, liver, and lungs.	Tubercle bacilli in glands and liver.	
14	47 days	1	0.9	Died, 64 days	Tubercle in inguinal and retroperitoneal glands, spleen, and liver.	do	Died 24 hours after injection of 0.1 c. c. tuberculin.
15	do	1	0.5	do	do	do	Do.
16	54 days	1		Died, 5 days	Peritonitis		
17	do	1		Died, 64 days	Tubercle in inguinal and retroperitoneal glands, spleen, and liver.	Tubercle bacilli in glands and spleen.	
18	63 days	1	1.1	Died, 57 days	do	Tubercle bacilli in glands and liver.	Do.
19	do	1	0.8	do	do	do	
20	70 days	1	0.8	Died, 65 days	do	Tubercle bacilli in glands	Do.
21	do	1	0.5	do	do	do	Do.
22	77 days	1	0.9	do	Tubercle in inguinal and retroperitoneal glands and spleen.	do	Do.
23	do	1	1.0	do	do	do	Do.
24	84 days	1	1.3	Killed, 40 days	Tubercle in inguinal and retroperitoneal glands.	do	0.1 c. c. tuberculin inoculated.
25	do	1	1.5	do	do	do	Do.
26	104 days	1	2.0	Killed, 70 days	Tuberculosis in inguinal glands	do	Do.
27	do	1	0.3	do	All organs and glands normal; much increase in weight.	No tubercle bacilli	Do.
28	125 days	1	0.4	do	All organs and glands healthy	do	Do.
29	do	1	0.2	do	do	do	Do.
30	132 days	1½	0.2	do	do	do	Do.
31	do	1½	0.5	Killed, 71 days	do	do	Do.

EXHIBIT III.—WHEY.

	Age and kind of whey.	c. c.					
1	Whey from cheese A, 4 hours old.	1.5	1.5	Killed, 70 days	Tubercle in inguinal and retro-peritoneal glands, spleen, and liver.	Tubercle bacilli in glands and spleen.	0.05 c. c. tuberculin inoculated.
2	Whey from cheese A, 48 hours old.	1.5	1.4	Killed, 49 days	Tubercle in inguinal and retro-peritoneal glands and spleen.	Tubercle bacilli in glands	0.1 c. c. tuberculin inoculated.
3	do	1.5	1.7	do	do	do	
4	Whey from cheese B, 4 hours old.	1.5	1.6	Died, 43 days	Tubercle in inguinal and retro-peritoneal glands, spleen, and liver; badly infected.	Tubercle bacilli in glands and liver.	0.05 c. c. tuberculin injected; died 24 hours later.
5	Whey from cheese B, 48 hours old.	1.5	1.1	Died, 49 days	Tubercle in inguinal and retro-peritoneal glands, spleen, and liver.	do	0.1 c. c. tuberculin inoculated; died 36 hours later.
6	do	1.5	1.4	do	do	do	

EXHIBIT IV.—CREAM CHEESE.

	Kind of cheese.	Grams.					
1	Cream cheese	2	1.2	Died, 57 days	Tubercle in inguinal and retro-peritoneal glands, spleen, and liver.	Tubercle bacilli in glands and spleen.	0.1 c. c. tuberculin injected; died 36 hours later.
2	do	2		Died, 4 days	do	do	Do.
3	do	2	0.3	Killed, 58 days	All organs and glands normal	No tubercle bacilli	0.1 c. c. tuberculin injected.
4	do	2	0.4	do	do	do	
5	do	2	0.2	do	do	do	Do.
6	do	2	0.2	do	do	do	Do.
7	do	2	1.3	do	Tubercle in inguinal and retro-peritoneal glands and spleen.	Tubercle bacilli in glands	Do.
8	do	2	1.2	Killed, 49 days	do		
9	do	2	2.1	Killed, 50 days	Tubercle in inguinal and retro-peritoneal glands.	Tubercle bacilli in glands	Do.
10	do	2	2.5	do	do	do	

Results of inoculating guinea pigs with cheese and whey of various ages, to test infection of tubercle bacilli—Continued.

EXHIBIT V.—CHEDDAR CHEESE MADE IN CANADA.

No. of guinea pig.	Age of inoculated cheese.	Quantity of cheese inoculated.	Tuberculin reaction.	Died or killed, and days after inoculation.	Postmortem.	Microscopic examination.	Remarks.
		Grams.	° *C.*				
1	Fresh curd	1	1.9	Died, 70 days	Tuberculosis of inguinal retroperitoneal glands, spleen, and liver.	Tubercle bacilli in glands	Died 18 hours after injection of 0.1 c. c. tuberculin.
2	do	1	1.4	do	do	do	Do.
3	7 days	1.5		Died, 6 days	Peritonitis	Coli infection	
4	do	1.5	1.5	Died, 78 days	Tuberculosis of glands, spleen, and liver; the latter badly infected.	Tubercle bacilli in glands	Died 24 hours after injection of tuberculin.
5	14 days	1.5		Died, 90 days	Tuberculosis of inguinal, retroperitoneal, and mesenteric glands; spleen and liver badly infected.	Tubercle bacilli in liver and retroperitoneal glands.	
6	do	1.5	2.2	Died, 84 days	do	do	Died 18 hours after injection of 0.1 c. c. tuberculin.
7	21 days	1.5		Died, 42 days	Tuberculosis of inguinal, retroperitoneal, and mesenteric glands, spleen, and liver.	Tubercle bacilli in liver	
8	do	1.5	1.4	Died, 63 days	do	Tubercle bacilli in liver and retroperitoneal glands.	Died 28 hours after injection of 0.1 c. c. tuberculin.
9	28 days	1.5		Died, 2 days	Peritonitis; edema	Coli bacteria and a bacillus resembling *B. edematis maligni.*	
10	do	1.5	1.7	Died, 63 days	Tuberculosis of glands, liver, and spleen.	Tubercle bacilli in liver	Do.
11	37 days	2	1.9	Killed, 65 days	Tuberculosis of inguinal and retroperitoneal glands and spleen.	Tubercle bacilli in glands	0.1 c. c. tuberculin injected.
12	do	2	1.6	do	do	do	Do.
13	42 days	2	1.6	Killed, 56 days	do	Tubercle bacilli in retroperitoneal glands.	Do.
14	do	2		Died, 12 days	Streptococcus infection	Streptococci in organs and blood.	

15	52 days	2	1.8	Killed, 63 days	Tuberculosis of inguinal and retroperitoneal glands; spleen slightly infected.	Tubercle bacilli in retroperitoneal glands.	Do.
16	do	2	1.7	do	do	do	Do.
17	62 days	2	1.7	Killed, 67 days	Tuberculosis of inguinal and retroperitoneal glands.	Tubercle bacilli in glands	Do.
18	do	2	1.5	do	Tuberculosis of inguinal glands	No tubercle bacilli	Do.
19	70 days	2	.6	Killed, 70 days	All organs and glands normal and healthy.	do	Do.
20	do	2	.4	do	do	do	Do.
21	80 days	2	.4	Killed, 64 days	do	do	Do.
22	do	2	.3	do	do	do	Do.
23	91 days	2	.4	do	do	do	Do.
24	do	2	.6	do	do	do	Do.
25	102 days	2	.4	Killed, 62 days	do	do	Do.
26	do		.4	do	do	do	Do.
27	112 days		.6	Killed, 82 days	do	do	Do.

If we examine Tables I and II we notice, almost at the first inspection, the difference in the vitality of the germs of tuberculosis taken from the different cheeses. For instance, in the case of guinea pigs inoculated with Emmenthaler cheese 14 days old, the tuberculin reaction was high, the animals did not succumb to the injection, and the post-mortem revealed less disease than was present in animals injected with Cheddar cheese 40 days old; and, compared directly with the animals injected with the latter cheese of the same age, namely, 14 days, the difference was still more marked, the guinea pig dying naturally of the disease, and the other dying from the inoculation with tuberculin. Like results may be noticed from all the succeeding inoculations.

OBSERVATIONS UPON THE RESULTS.

The tubercle bacilli in Emmenthaler cheese were all dead when the cheese was between 33 and 40 days old, for we find that the animals injected with 33-days-old cheese, although they gave a tuberculin reaction of 2° C. and over, showed no signs of the disease at the post-mortem, and both had gained considerably in weight. Cases similar to this have been noticed in tuberculin-tested cattle. Probably, if the inguinal glands from these animals had been transferred to other guinea pigs, the disease might have established itself in the reinjected animals. The animals subsequently injected (40 days, 47 days, etc.) were all free from tubercle and gave no marked tuberculin reaction. Two animals, however, died from other causes.

The tubercle bacilli in the Cheddar-infected cheese were more long-lived, as they continued capable of causing disease up to 104-days-old cheese, at which time one guinea pig had a very slight infection and the other was quite free from tubercle. By a mistake the guinea pigs infected from cheese 111 and 113 days old, respectively, were killed for other work about a month after injection. They were all perfectly healthy; but as they had not been tested for tuberculin, and as sufficient time had not been given for tubercle bacilli to establish themselves, they are not included in Table II. One may, however, safely conclude that the vitality of tubercle bacilli in this cheese did not much, if any, exceed 104 days.

The great difference in the duration of life of the germs in the two cheeses—about 70 days—can be ascribed only to the difference in the method of manufacture. The heat used in making the Emmenthaler cheese (from 50° to 54° C.) and the length of time the curds are kept at this temperature evidently causes the death of the weaker tubercle bacilli and the enfeeblement of the more resistant. The whey from this cheese was also less virulent. Guinea pigs inoculated with it did not die after the tuberculin inoculation, and the post-mortem showed less disease than in those animals inoculated with the same quantity of whey from the Cheddar cheese.

The guinea pigs inoculated with cheese (both kinds) 7 days old all died two days after inoculation, and from these animals the bacillus of malignant edema, as well as *Bacillus coli* and *B. staphylo-albus*, were isolated. I can not believe that the presence of these germs was due to an accidental infection, as exceptional precautions were taken to avoid such complications. At the same time it is remarkable that subsequent inoculations did not show the presence of the bacillus of malignant edema, which, on account of the resistant nature of its spores, one would expect to survive in the cheese for some time. Weigmann has found and cultivated from cheese bacilli similar in morphological character.

The tuberculin reactions which served incidentally for measuring the strength of the tuberculin used have been already mentioned, and the results obtained fully confirm those given by Dönitz. They also served, apart from the post-mortem, to give some idea of the extent of the disease in the guinea pigs, the amount of the disease usually being in inverse ratio to the amount of the reaction. Reference and comparison of Tables I and II will show this very strikingly.

CONCLUSIONS FROM THE ORIGINAL EXPERIMENTS IN SWITZERLAND.

From these results we are safe in concluding that hard cheese—especially Emmenthaler—is quite safe for human food so far as tuberculosis is concerned. Both Cheddar and Emmenthaler are seldom eaten until they are 4 months old or more; at this age all tubercle bacilli which they may have contained will be dead. We should also remember that the number of tubercle bacilli likely to be present in cheese as commonly made is very small, and that in all probability those will die sooner than bacilli put into cheese in the course of experiments like these. Besides this, the original number of such germs in milk will be reduced by being carried off in the whey.

From the experimental data it would seem advisable to use the milk of tuberculous cows, segregated according to Bang's system, for making cheese rather than butter—that is, on the assumption that the latter is made without proper pasteurization of milk or cream. The whey from such milk should be heated to 85° C. (185° F.), which would not only kill any tubercle bacilli that might be present, but would also have other excellent economic results.

EXPERIMENTS WITH WHEY, AND COMMENTS.

The experiments with the whey from cheeses A and B were only to find out if the tubercle bacilli in milk were present in numbers in the whey and if they could live therein for 48 hours, as this is about the limit of time that whey is kept. As has already been remarked, the whey from the Cheddar cheese (B) was more virulent than that from

the Emmenthaler (A), but the latter was also infectious. Ostertag[4] has noted that intestinal tuberculosis is sometimes caused by feeding separator slime to pigs; Galtier has also remarked on the probability of swine and poultry becoming infected with tubercle by eating the by-products of milk; and, in my opinion, this phase of the question is even more important than the human side. At present, outside of Denmark, very little whey is pasteurized, or rather heated to 85° C.; and when we consider the small cost of the operation (as exhaust steam may be used for the purpose) and the benefits to be derived from it, we wonder why it is not done more frequently. In America cheese makers look upon the contamination of milk cans by unpasteurized whey as one of the principal sources of the many troubles met with in making cheese during the summer months. So we have a threefold benefit from heating whey to 85° C.: (1) Destruction of tubercle and other pathogenic bacteria; (2) better keeping of the whey itself; (3) the removal of a prolific source of bacterial contamination of milk cans.

Denmark[5] recognizes the danger of the by-products of milk and has enacted a law which makes compulsory the heating of all such products (whey, skim milk, and buttermilk) to at least 85° C. (185° F.).

TRIALS WITH CREAM CHEESE, AND RESULTS.

As will be noticed in the tables, a few trials were also made with cream cheese. The samples of this variety were bought on the open market in Berne, and the age of some of them was not known; but they all appeared to be only a few days old. Too few samples were examined to warrant the drawing of general conclusions from the experiments. The amount of cheese injected was rather large; and we must remember that a larger proportion of tubercle bacilli is found in cream and in milk sediment than in the milk. Even in the separator many bacilli are thrown out with the cream. So we may expect that a larger number will be found in cream cheese than in ordinary hard cheese. Of the five samples examined, three were found to contain tubercle bacilli—one cheese evidently containing large numbers, if we can judge from the amount of infection. (See guinea pig No. 1, Table IV). The others were only slightly infected.

There is evidently, then, some danger to be apprehended from cheese of this kind—just how much can not be stated. But there is no reason why pasteurized cream should not be used for cream cheeses, because the objections to using pasteurized milk for making hard cheese do not hold in making cream cheese.

NOTE.—The work described herein was done in the bacteriological institute of the University of Berne, and I wish to record my sincere thanks to Dr. de Freudenreich for his kind assistance, interest, and criticism while the work was in progress.—F. C. H.

SUPPLEMENTARY REPORT BASED UPON CHEDDAR CHEESE.

The experiments upon the duration of life of the tubercle bacillus in cheese made at Berne in 1899–1900 (published in the Landwirtschaftlichen Jahrbuch der Schweiz, 1900, and of which the foregoing is a translation), demonstrated that the tubercle bacillus died out between the thirty-third and fortieth days in cheese made after the Emmenthaler method; but in cheese made approximately after the Cheddar method the duration of life of the bacillus was considerably longer.

On account of objections to the imperfect method of manufacture of the Cheddar-like cheese made in Berne, it seemed advisable to repeat the experiment by making a typical Cheddar cheese in a country where such cheese is regularly made. As Russell remarks (Outlines of Dairy Bacteriology):

> Our domestic Swiss cheese, or even cheese of this class made in Germany, rarely have the peculiar flavor that is found in the product imported from the Swiss valleys. For centuries this brand of cheese has been made in that country, until the factories and dairies have become stocked with the right kind of germs, capable of producing the desired fermentation.

If this is true of Swiss cheese manufactured in America, it is likely to be also true of Cheddar cheese made in Switzerland. If added to this objection there is the further one that the details of manufacture of Cheddar cheese made in Switzerland were not identical with the ordinary practice in the making of this cheese, we clearly see that in the interest of scientific accuracy, as well as in the comfort and security that might be obtained from the knowledge of the fact that any tubercle bacilli were likely to be dead before the cheese was ripe and ready to be eaten, it was necessary to carry out another series of experiments upon Cheddar cheese made in the approved manner. This was accordingly done by the writer in the year 1901, and these experiments are first described and reported as follows:

CULTURE.

The culture used was of bovine origin, the seventh transfer since isolation from a tuberculous guinea pig inoculated with a piece of liver from a tuberculous cow. The whole of the growth from 700 c. c. of glycerin bouillon 12 weeks old was used. The growth was very heavy, and on account of the difficulty of separating the masses of bacilli in the pellicle, sterilized powdered glass was used in order to break up the clumps of bacilli, and a fairly good emulsion was thus obtained, which was added to the milk at the same time as the rennet. The infected milk was then stirred constantly for five minutes, when coagulation commenced.

THE CHEESE.

Eighty pounds of milk in good condition were used for making the cheese. The acidity of the milk was 0.14 per cent, and 1½ pounds of a pure culture of a lactic acid baccillus was added, together with 1 dram of cheese color. The milk was set at 86° F., after the rennet test was found to be twenty seconds. Two drams of rennet were used. The other particulars about making this cheese were as follows:

Time between setting and dipping	3 hours.
Time between dipping and milling	2 hours 10 minutes.
Time between milling and salting	1 hour 10 minutes.
Amount of acid at milling	1½ inches.
Amount of salt	3 ounces.
Acidity of curd before salting	8 per cent.
Weight of press on cheese	1,000 pounds.
Weight after cheese was turned	2,000 pounds.

The cheese worked quite normally, the curd having a nice silky feeling and meaty texture. All operations of stirring, etc., were performed with the hands inclosed in a pair of rubber gloves, such as are employed for surgical use, the endeavor being to produce a typical Cheddar cheese, made in exactly the same manner as in a cheese factory.

The cheese was ripened at an average temperature of 60° F., which is regarded as being very suitable for Cheddar cheese, as it does not injure the texture or cause the fat to run out.

With regard to such details as the amount of cheese used for inoculation of the guinea pigs, the method of inoculation, observations of animals during life, autopsy, and microscopic preparations, they were the same as in the Berne experiments; hence it is unnecessary to repeat them.

The acidity of the cheese was tested when a month old. Five grams of cheese and an equal amount of glass were ground together in a mortar; 100 c. c. of water was then added and well mixed with the cheese. After standing fifteen minutes, the mixture was passed through a filter paper. Twenty-five cubic centimeters of the clear filtrate were taken for the determination of the acidity. Phenolphtalein was used as an indicator. The result of this test showed 0.95 per cent acid—figured as lactic acid. A bacteriological analysis was also made at the end of one month; and 1 gram of cheese contained 43,700,000 lactic acid bacteria. No other species were present on the culture plates.

Reference to the table shows that the tubercle bacilli died out somewhere between the sixtieth and sixty-second days. Even previous to the sixty-second day there was evidence to show that the number of living tubercle bacilli was small, or else their virulence was much

weakened, for the guinea pigs inoculated on the forty-second and fifty-second days were all lightly infected.

Three animals were lost from other infection. The one which died after the inoculation of cheese 7 days old may have obtained the infection directly from the cheese, but the other two cases were probably due to other causes.

A comparison of the results obtained from this true Cheddar cheese with the results of the partly simulated Cheddar made at Berne show that considerable difference exists between the two; in fact, a difference of thirty-four days in favor of the genuine Cheddar. There are several probable explanations of this difference. The greater acidity developed in the true Cheddar cheese, both during making and subsequent ripening, must have a certain effect on the tubercle bacilli, and the fact that the salt was directly mixed with the curd, instead of being rubbed on the cheese from the outside and then slowly penetrating inward, would also have some influence. The pressure on the properly made cheese was also much greater, and although this would make no difference to the bacilli present in the cheese, a much closer texture resulted, and this may have had some effect. All three of these factors might together influence and curtail the duration of life of the tubercle bacilli in Cheddar cheese.

CONCLUSIONS VERIFIED AND REPEATED.

These later experiments fully verified the conclusions first reached and justify this statement: If Cheddar cheese, as commonly made in the United States and Canada, happens to contain tubercle bacilli, naturally present, it may be assumed that none of these germs will be living when the cheese becomes ten weeks old; hence no danger need be apprehended of acquiring the disease known as consumption by eating well-cured Cheddar cheese.

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